APPLICATION FOR

SYNTHESIS OF A MIXTURE OF SULFATED ESTROGENS

USING A SULFUR TRIOXIDE COMPLEX

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Synthesis of a Mixture of Sulfated Estrogens Using a Sulfur Trioxide Complex

Field of the Invention

[0001] The present invention relates to a process for the synthesis of a mixture of sulfated estrogens which may comprise sulfated $\Delta^{8,9}$-dehydroestrone, estrone, equilin, and derivatives thereof, among others.

Background

[0002] Naturally occurring estrogenic compositions are used in medical treatments to alleviate the symptoms of menopausal syndrome and osteoporosis/osteopenia in estrogen deficient women, prevent cardiovascular disease in men and women, and treat other hormone related disorders. The estrogenic components of the naturally occurring estrogenic compositions include sulfate esters of estrone, as disclosed in U.S. Patent No. 2,834,712.

[0003] The synthesis of sulfated estrogens has been described in past publications. For example, U.S. Patent No. 5,288,717 to Raveendranath et al. (Alkali Metal 8,9-Dehydroestrone Sulfate Esters) teaches a process of synthesizing alkali metal salts of 8,9-dehydroestrone ($\Delta^{8,9}$-DHE) and its sulfate ester free from other conjugated esters present in material found in natural sources of mixed esters. In the process of Raveendranath, an alkali metal salt of $\Delta^{8,9}$-DHE is initially produced followed by sulfation with sulfur trioxide-trimethylamine complex (SO_3-TMA) under mild conditions in an apolar, aprotic solvent such as tetrahydrofuran (THF) with simultaneous or subsequent addition of tris(hydroxymethyl)aminomethane (TRIS) as a stabilizer. The alkaline bases employed in the production of the initial intermediates of $\Delta^{8,9}$-DHE are preferably sodium or potassium in the form of their hydrides and lithium as n-butyl lithium. This process provides a product free of other conjugated esters and does not

teach production of several compounds at once.

[0004] U.S. Patent No. 5,998,639 to Raijmakers, et al. (Sulfatation of Estrogen Mixtures) teaches a process for the preparation of a mixture of sulfated estrogens containing $\Delta^{8,9}$-DHE or derivatives thereof. In the process of Raijmakers, an estrogen mixture is obtained by isomerization of equilin or a derivative thereof using lithium salts of ethylene diamine. This mixture is sulfated with sulfuric acid/acetic anhydride/pyridine. The mixture of crude pyridinesulfates is treated with sodium hydroxide in methanol, yielding a mixture in a specific ratio of $\Delta^{8,9}$-DHE sodium sulfate and one or more of, for example, equilin sodium sulfate, 17α-dihydro equilin sodium sulfate, 17β-dihydro equilin sodium sulfate, 17α-estradiol sodium sulfate, and 17β-estradiol sodium sulfate.

[0005] There remains a need for an efficient process of producing a stable composition of a mixture of sulfated estrogens.

Summary of the Invention

[0006] The present invention provides processes for the production of stable compositions comprising complex mixtures of sulfated estrogens. Previous synthetic procedures have involved synthesis of an estrogen, or a mixture of estrogens, from synthesis of a precursor. The present invention provides for the synthesis of complex estrogens by parallel synthetic processes on a mixture of precursors.

[0007] In one aspect, the estrogens can comprise at least two of $\Delta^{8,9}$-DHE, estrone, equilin, or derivatives thereof. The mixture of sulfated estrogens would correspondingly comprise sulfated alkali metal salts of $\Delta^{8,9}$-DHE, estrone, equilin, or derivatives thereof. These compounds are obtained in ratios not obtained

when synthesized individually. The process comprises reacting a sulfur trioxide complex with a mixture of alkali metal salts of estrogens; adding a stabilizing amount of TRIS; and recovering the stable composition comprising the mixture of sulfated estrogens and TRIS. The process may further comprise reacting a mixture of estrogens with an alkali metal hydride to provide the mixture of alkali metal salts of estrogens. The process may be performed in an apolar, aprotic solvent. All steps of the process may also be performed in a single reaction vessel.

[0008] One advantage of the present invention is that the process produces a mixture of sulfated estrogens in a single vessel. In this respect, the mixture of alkali metal salts of $\Delta^{8,9}$-DHE, estrone, equilin, and/or related substances is sulfated simultaneously to provide a complex mixture of sulfated estrogen alkali metal salts having a potentially altered estrogenic composition and with ratios of the three primary estrogens that would not have been produced if synthesized individually. Furthermore, the entire reaction sequence of the process may be performed in a single vessel without isolating intermediate products.

[0009] In another aspect of the present invention, the process produces a mixture of sulfated estrogens in a specific ratio. By way of example, a mixture of estrogens comprising a specific ratio of $\Delta^{8,9}$-DHE, estrone, equilin and derivatives thereof is provided. The process of the present invention is performed on this mixture of estrogens and produces a mixture of sulfated estrogens in the same approximate ratios as that of the starting estrogens.

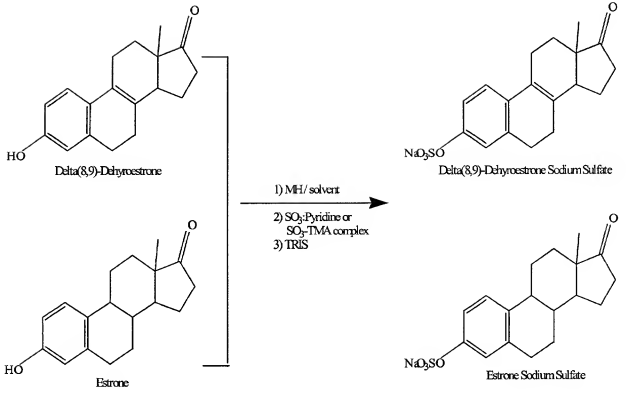
Detailed Description of the Illustrative Embodiments

[00010] According to the invention, a mixture of sulfated estrogens is

produced, preferably using a single vessel. In the process of the present invention, a mixture of alkali metal salts of estrogens may be prepared from a first mixture of estrogens. Typically, the first mixture will contain at least two estrogens. The estrogens may be any estrogenic compound, including $\Delta^{8,9}$-DHE, estrone, equilin, 17α-estradiol, l7β-estradiol, 17α-dihydroequilin, 17β-dihydroequilin, equilenin, l7α-dihydroequilenin, 17β-dihydroequilenin, 17α-$\Delta^{8,9}$-dehydroestradiol, 17β-$\Delta^{8,9}$-dehydroestradiol, 6-OH equilenin, 6-OH 17α-dihydroequilenin, 6-OH 17β-dihydroequilenin, ethinyl estradiol, and estradiol valerate, and derivatives thereof. Derivatives thereof, as used herein includes any compounds derived from or related to the estrogenic compounds named herein.

[00011] The mixture of alkali metal salts of estrogens may be prepared by reacting the mixture of estrogens with an alkali metal hydride in an apolar, aprotic solvent. The mixture of alkali metal salts of estrogens may be sulfated using a sulfur trioxide complex in an apolar, aprotic solvent. For stability, an amount of TRIS may be added to the mixture of sulfated estrogens.

[00012] Accordingly, the general synthetic scheme as it applies to estrone and $^{\Delta 8,9}$DHE is of the present invention is as follows:



[00013] According to the general synthetic scheme of the present invention, a mixture of estrogens are reacted with an alkali metal hydride (MH), including for example, NaH, KH, LiH, and the like. This reaction may be performed in an apolar, aprotic solvent, including for example, THF, dioxane, diethyl ether, and the like. Where the mixture of estrogens comprises $\Delta^{8,9}$-DHE, estrone, and derivatives thereof, this reaction produces a mixture of alkali metal salts of estrogens comprising alkali metal salts of $\Delta^{8,9}$-DHE, estrone, and derivatives thereof.

[00014] The mixture of alkali metal salts of estrogens is reacted with a sulfur

trioxide complex, including for example, SO_3-TMA, SO_3-pyridine, and the like. This reaction also may be performed in an apolar, aprotic solvent, including, THF, dioxane, diethyl ether, and the like. Where the mixture of estrogens comprises $\Delta^{8,9}$-DHE, estrone, and derivatives thereof, this reaction produces a mixture of sulfated alkali metal salts of estrogens comprising alkali metal salts of $\Delta^{8,9}$-DHE, estrone, and derivatives thereof.

[00015] To the mixture of sulfated estrogens is added a stabilizing amount of TRIS. These three reaction steps may be performed sequentially in a single reaction vessel without isolating the intermediate products. The composition comprising the mixture of sulfated estrogens and TRIS is recovered. As one of skill in the art would readily recognize, the composition may be recovered by any number of ways, including, for example, filtration, extraction, and the like. The resulting product may also be purified by any number of purification techniques, also well known in the art, including, for example, recrystallization, chromatography, and the like.

[00016] The synthetic scheme of the invention may be applied to any mixture of estrogens or their derivatives. These mixtures typically will comprise at least two estrogens or corresponding alkali metal salts of the estrogens.

Examples

[00017] The invention will be further explained by the following illustrative examples that are intended to be non-limiting.

Example 1

[00018] Sodium hydride (NaH) (0.77 g, ~0.0304 mole) and THF (60mL) under nitrogen atmosphere were added to a dry, 500 mL, three-neck, round bottom

flask equipped with an air condenser, a 100 mL addition funnel, and magnetic stir bar. The suspension was stirred and cooled to 0-5°C. Next, $\Delta^{8,9}$-DHE (5.11g, ~0.0187 mole) dissolved in 75 mL THF, and solid estrone (1.27g, ~0.0046 mole) were added at 0-5°C under a nitrogen atmosphere. After 30 minutes, the cooling bath was removed to allow the reaction mixture to attain ambient temperature, and the mixture was stirred for 2-2.5 hours at 20-22°C. Sulfur trioxide-pyridine complex (SO_3-Pyridine) (4.05g, ~0.025 mole) was added in small batches to the reaction mixture. After stirring for 30 minutes, TRIS (2.82 g, ~0.0233 mole) was added, and stirring was continued overnight at 20-22°C under a nitrogen atmosphere. The mixture was transferred into a 1 L round bottom flask. The solvent was evaporated under high vacuum (0.15 mm of Hg pressure) at 20°C, and then the pyridine was removed at 29-30°C. This step was repeated by adding 20 mL of fresh THF to the residue. The residue was taken up in 130 mL of deionized water, and the aqueous solution extracted nine times with 50 mL of diethyl ether to remove unreacted $\Delta^{8,9}$-DHE and estrone. To the 130 mL of the aqueous solution were added 90 mL of deionized water and 1.6 g of activated carbon, "Darco." This was stirred for 30 minutes and filtered through a sintered glass funnel using filter agent "Celite-521." The carbon treatment was repeated by adding 1.6 g of "Darco," stirring for another 30 minutes, and filtering through a sintered glass funnel using filter agent "Celite-521." The TLC (thin-layer chromatography) of this solution [$CHCl_3$:MeOH:NH_4OH (25:5:1)] did not show a spot corresponding to $\Delta^{8,9}$-DHE. The filtrate was lyophilized to obtain 9.1 g of light tan colored solid. The sample was analyzed by HPLC (high performance liquid chromatography), weight % process.

Results

Yield	= 9.1 g
HPLC Wt. % assay: DHES %	= 42.8%
ES %	= 12.2%
Total % HPLC wt. % Assay (DHES + ES)	= 55.0
Molar Ratio of DHES:ES	= 1:0.29
Molar Ratio of (DHES + ES):TRIS	= 1:1.73
Moisture content by Karl-Fisher	= 3.2%

Example 2

[00019] The general process described in Example 1 was followed, except that SO_3-Pyridine was replaced by SO_3-TMA, and resultantly, the trimethylamine, as opposed to the pyridine, was removed.

[00020] Starting materials and reagents:

Compound (purity)	Weight	Molar Amount
NaH (95%)	0.64 g	~0.0253 mole
$\Delta^{8,9}$-DHE (98%)	5.02 g	~0.0183 mole
Estrone (99%)	1.22 g	~0.0045 mole
SO_3-TMA (98%)	3.66 g	~0.0258 mole
TRIS (99.9%)	2.82 g	~0.0233 mole

Results

Yield	= 8.1 g
HPLC Wt. % assay: DHES %	= 44.70%
ES %	= 9.54%
Total HPLC wt. % assay(DHES + ES)	= 54.24

Molar Ratio of DHES:ES	= 1:0.21
Molar Ratio of (DHES + ES):TRIS	= 1:1.96

Example 3

[00021] The general process described in Example 2 was followed.

Starting materials and reagents:

Compound (purity)	Weight	Molar Amount
NaH (95%)	2.01 g	~0.0793 mole
$\Delta^{8,9}$-DHE (98%)	10.0 g	~0.0366 mole
Estrone (99%)	2.55 g	~0.0093 mole
SO_3-TMA (98%)	11.5 g	~0.0811 mole
TRIS (99.9%)	8.47 g	~0.0699 mole

Results

Yield	= 23.79 g
HPLC Wt. % assay: DHES %	= 31.5%
ES %	= 9.1%
Total HPLC wt % assay(DHES + ES)	= 40.6
Molar Ratio of DHES:ES	= 1:0.30
Molar Ratio of (DHES + ES):TRIS	= 1:2.69

Summary of Results

Exp.	Molar Ratios of Starting Materials				DHES %	ES %	Results		% Yield
							Molar Ratios		
	DHE + E	NaH	SO_3-Amine	TRIS			DHES:ES	(DHES + ES):TRIS	
1	1	1.30	1.07	1.00	42.8%	12.2	1:0.29	1:1.73	55.00
2	1	1.11	1.13	1.02	44.7%	9.54	1:0.21	1:1.96	54.24
3	1	1.77	1.73	1.52	31.5%	9.1	1:0.30	1:2.69	40.60

Example 4

[00022] Chromatograms were compared to illustrate the differences between the processes taught herein and methods producing only one estrogenic compound at a time. Each chromatogram was obtained with the same chromatographic procedures. The chromatograms are detailed in Table 1 below which provides the counts per peak over time for the production of each of estrone, equilin, $\Delta^{8,9}$-DHE (Delta-8,9), and a combination of these compounds produced by the processes of the invention (3-Combi). The table also provides ratios at each time indicated comparing each individual compound to the combination of estrogens prepared as taught herein. These relative ratios show the distinct differences in the results of the individual processes versus the combined process. As illustrated in the table, the processes of the invention enable the production of different ratios of estrogen products than can be obtained by preparing estrogenic compounds separately.

Table 1

Time (min)	Counts/Peak				Ratio		
	Estrone	Equilin	Delta-8,9	3-Combi	Estrone: 3-Combi	Equilin: 3-Combi	Delta-8,9: 3-Combi
25.721	0.50	0.50	0.50	3.05	0.16	0.16	0.16
24.984	0.50	0.50	0.50	6.15	0.08	0.08	0.08
22.152	0.50	53.81	0.50	139.62	0.00	0.39	0.00
21.975	0.50	0.50	0.50	32.07	0.02	0.02	0.02
21.634	0.50	0.50	0.50	4.33	0.12	0.12	0.12
20.237	0.50	0.50	204.47	41.58	0.01	0.01	4.92
19.436	0.50	0.50	10.50	31.66	0.02	0.02	0.33
18.722	4.08	0.50	0.50	31.13	0.13	0.02	0.02
18.376	0.50	0.50	15.00	74.69	0.01	0.01	0.20
15.818	0.50	15.51	1.00	3.27	0.15	4.74	0.31
12.591	0.50	0.50	3.91	18.93	0.03	0.03	0.21
11.944	0.50	0.50	15.75	2.78	0.18	0.18	5.67
10.981	0.50	0.50	0.50	4.22	0.12	0.12	0.12
10.373	0.50	0.50	0.50	2.68	0.19	0.19	0.19
10.222	0.50	0.50	0.50	2.18	0.23	0.23	0.23
9.910	0.50	0.50	8.57	3.33	0.15	0.15	2.58
8.901	0.50	0.50	0.50	4.01	0.12	0.12	0.12

[00023] Various modifications and alterations of this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention.